Original Paper

Isolation and Identification of Diarrhea Causing \textit{Shigella} species Using Various Biochemical and Molecular Methods

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Abstract

The global morbidity & mortality due to \textit{Shigella} species is one of the major public health problems that accounts for thousands of deaths among the children's below 5 years of age in many developing countries including Bangladesh. The present study was designed to isolate and characterize \textit{Shigella} like organisms (Non-lactose fermenting colonies) by allowing biochemical characteristics, serology and PCR, from the stools of clinically Shigellosis patients. A total of 20 stool samples were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). All of these cultures were further analyzed by using different molecular tools. Cultures were screened for \textit{ipa}H gene which is specific for all \textit{Shigella} species. Out of the 20 enriched stool samples, 3 samples were positive for \textit{ipa}H gene. After performing serological analysis, 2 samples were confirmed as \textit{Shigella flexneri} and one as \textit{Shigella sonnei}, In addition, PCR was done for 3 serologically confirmed samples.

Keywords: PCR, serology, biochemical tests, \textit{ipa}H gene.

Introduction

Diarrheal diseases are one of the fatal diseases in Bangladesh and other developing countries. \textit{Shigellosis} is one of the major diarrheal diseases in these countries, which causes significant number of deaths especially among the children [1]. About 1.1 millions of people were estimated to die from \textit{Shigella} infection each year, with 60\% of the deaths occurring in children under 5 years of age. More recent estimates fix the \textit{Shigellosis} disease burden at 90 million episodes and 1080000 deaths per year [2]. \textit{Shigellosis} is endemic throughout the world where it is held responsible for some 120 million cases of severe dysentery with blood and mucus in the stools and ulceration in the intestinal epithelium. The World Health Organization has emphasized the need to understand the disease burden and the epidemiology of \textit{Shigella} infections in developing countries [3]. Determining the fact that \textit{Shigella} is the cause of the illness depends on laboratory tests that identify the bacteria in the stool of an infected person. Some of the tests may not be performed routinely, so the bacteriology laboratory should be instructed to look for the organism. The laboratory can also do tests to determine which type of \textit{Shigella} is involved and which antibiotics would be best for treatment.

Materials and Methods

To carry out the complete detection method of \textit{Shigella}, biochemical, serology and PCR investigations were done with 20 stool samples of patients. Clinical detection was carried out for 4 weeks (Feb. 2013 to March 2013) to complete detection procedures in International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Samples were streaked on MacConkey Agar plate and incubated at 37°C for 18 hours. After overnight incubation, typical \textit{Shigella} colonies, white or colourless identified on
the selective agar plates were confirmed by short biochemical tests. The biochemical reactions of the strains were determined by standard methods described in manual for laboratory investigation of acute enteric infections [4]. Following biochemical tests were performed: Kliger’s iron agar (KIA) test, Motility indole urea (MIU) test, Simon’s Citrate agar (SCA) test, Sodium acetate utilization, Lysine decarboxylase, Arginine dihydrolase, Ornithine decarboxylase test, Triple sugar iron (TSI) test and Carbohydrate fermentation test. The biochemical tubes were inoculated by stabbing the straight wire into the centre of the agar down to the base of the reaction tube. Then the wire is pulled back and the rest of the inoculum is distributed on the slope of the agar surface (KIA and citrate tube has slope). Isolates that were non-motile and didn’t produced H₂S and found to be negative for urease, oxidase, indole production were identified as Shigella spp. All the isolates were serologically confirmed by using commercially available antisera kit (Denka Saiken, Co. Ltd. Japan) specific for all group and type- factor antigens. Isolates were subcultured on MacConkey agar (Difco, Becton Dickinson & Company Sparks, MD, USA) plates and after about 18 h of incubation, serological reactions was performed by the glass slide agglutination test.

Each serotype has been given an antigenic formula according to its antigenic structure, which can be identified by agglutination tests using various specific sera. The isolates of different morphological patterns were further analyzed by PCR for confirmation of individual colonies being ipaH positive or not. Previously identified individual colonies were confirmed as ipaH positive [5, 6].

**Results and Discussion**

Out of the total 20 samples, 07 samples were taken for biochemical tests as per colony observation (white or colourless) and 04 showed positive results in biochemical tests. The genus *Shigella* are gram-negative, non-motile and non-spore forming bacteria. All these strains are non-lactose fermenting; non-motile. In Kliger’s Iron Agar (KIA) test, slant was alkaline and butt showed acidic condition. In Motility Indole Ornithine (MIO) test, all strain showed the non-motility pattern, ornithine is decarboxylated and indole negative characteristics. They neither utilized Simon’s citrate, nor produced H₂S and did not utilize urea or urease negative (Figure 1). Thus they are similar to *Shigella*. The results of biochemical analysis are shown in (Table 1).

**Table 1**: Biochemical analysis of suspected *Shigella*-like organisms (SLO)

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>KIA</th>
<th>MIU</th>
<th>Citrate</th>
<th>Lactose fermentation</th>
<th>Suspected organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample no.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>K</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>09</td>
<td>K</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>K</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>K</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Each serotype has been given an antigenic formula according to its antigenic structure, which can be identified by agglutination tests using various specific sera. The isolates of different morphological patterns were further analyzed by PCR for confirmation of individual colonies being ipaH positive or not. Previously identified individual colonies were confirmed as ipaH positive [5, 6].

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Isolation and Identification of Diarrhea Causing Shigella species

Advances in Biomedicine and Pharmacy Vol. 2 (6) 2015

Confirmation by PCR
After analyzing the suspected colonies by biochemical and serological analysis (Table 2), the identified colonies were further confirmed by PCR (figure 2).

Table 2: Serological analysis of isolated Shigella strain

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>ipaH gene</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 06</td>
<td>+</td>
<td>Shigella flexneri</td>
</tr>
<tr>
<td>Sample 09</td>
<td>+</td>
<td>Shigella sonnei</td>
</tr>
<tr>
<td>Sample 15</td>
<td>+</td>
<td>Shigella flexneri</td>
</tr>
</tbody>
</table>

Figure 1: KIA, MIU and citrate test result of typical Shigella spp.

Figure 2: PCR analysis of identified colonies for virulence gene ipaH. Products of PCR assay for the ipaH gene are shown. Lanes 1 through 19 shows the products of individual colony. Lane 15 represents negative control, 16 shows positive control and 19 shows 1 Kb plus DNA ladder.
Conclusion
This study confirms that the combination of culture method, biochemical tests, serology and PCR based molecular method is remarkably effective to determine the actual disease burden caused by *Shigella*. Therefore, this study has demonstrated good prospects of upcoming research to explore the actual disease burden of invasive diarrhea.

Acknowledgement
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Conflict of interest
The authors declare that there is no conflict of interest to reveal.

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References